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Genetic basis of salt-susceptibility in the Sabra rat model of hypertension

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Genetic basis of salt-susceptibility in the Sabra rat model of hypertension. The Sabra salt-sensitive SBH/y and salt-resistant SBN/y rats constitute a unique experimental model of hypertension in which salt-susceptibility is genetically determined and expressed only after salt-loading, without the development of spontaneous hypertension. To determine the genetic basis of salt-susceptibility in the Sabra rats, the candidate gene and total genome screen approaches were adopted. The likely candidate genes in this model incorporate salt-related physiological mechanisms such as the nitric oxide system, the arginine vasopressin axis and the epithelial sodium channel. In the random genome search scheme for culprit genes, SBH/y and SBN/y were cross-bred. A highly unusual and composite mode of transmission of salt-susceptibility was found in this cross, emphasizing the complexity of the genetic basis of salt-susceptibility. Linkage analysis of the entire rat genome with a large number of widely distributed microsatellite markers identified three putative gene loci on chromosomes 1 and 17 that contribute importantly to salt-sensitivity and/or resistance, and uncovered sex specificity in the role that salt-susceptibility genes fulfill in the development of hypertension.

In the complex, polygenic and multifactorial form of disease that comprises hypertension, high dietary salt intake remains one of the more important environmental factors that predispose individuals to the development of hypertension. Experimental and clinical data suggest that the individual's blood pressure (BP) response to salt loading, categorized as salt-sensitivity when BP rises and salt-resistance when BP is unaffected, is determined by salt-susceptibility genes [1]. The search for these susceptibility genes in humans has been hampered thus far by the inability to isolate this phenotype from the numerous other confounding elements that determine BP, such as the effects of dietary factors other than salt, age, body mass, gender, the multitude of environmental factors, and the involvement of additional genes that induce the development of "spontaneous" hypertension (Fig. 1). The closest one has been able so far to close in upon the specific genes for salt-susceptibility may have been by studying the Dahl experimental model of hypertension, one of the two major models of salt-induced hypertension. The Dahl rats were originally developed as a model of salt-sensitivity by L.K. Dahl and further inbred and developed by J. Rapp a decade ago [2, 3]. Breeding experi-

ments and cosegregation studies using molecular genetic tools in crosses between the Dahl-derived rats and other rat strains have revealed a relatively large number of BP-relevant quantitative trait loci (QTL), presumably incorporating the salt-susceptibility genes, on chromosomes 1, 2, 3, 5, 7, 9, 10, 13 and 17 [4–9]. Which of these quantitative trait loci relate to salt-susceptibility *per se*, however, is unclear because the Dahl rats, in addition to expressing salt-sensitivity and resistance, develop spontaneous hypertension as a function of age, even in the absence of dietary salt exposure [10]. It may thus not be possible to differentiate between QTLs that encode genes for salt-susceptibility from those that encode for spontaneous hypertension in the Dahl model of salt sensitivity. For this reason, the Dahl experimental model of hypertension, although being a classical model of spontaneous hypertension on which salt-susceptibility is genetically superimposed, which is in fact reminiscent of the clinical phenotype of hypertension in humans, may be less of an ideal experimental model for the study of the genetic basis of salt-susceptibility. A preferable experimental model would be one that expresses salt-susceptibility as an isolated BP phenotype, not confounded by spontaneous hypertension. Such is the Sabra model of salt-susceptibility, the second major model of salt-induced hypertension.

The Sabra model of hypertension was developed over two decades ago by D. Ben-Ishay and co-workers as a new rat model of salt-sensitive and salt-resistant hypertension that is genetically and phenotypically distinct from the Dahl strains [11, 12]. Recently, we undertook secondary inbreeding of the original colony, rendering the SBH/y and SBN/y animals phenotypically homogeneous and genetically highly suitable for studying the genetic basis for salt-susceptibility [13]. Phenotypically, the Sabra rat model of hypertension expresses hypertension only after salt-loading, without ever developing spontaneous hypertension [13]. This feature bestows upon the Sabra model an advantage insofar as the ability to study the genetic basis of salt-susceptibility without the confounding element of essential hypertension. Screening of the Sabra genome with microsatellite markers revealed a relatively high degree of polymorphism between the salt-sensitive SBH/y and salt-resistant SBN/y strains [13]. This important feature in the Sabra model allows cross-breeding between the two strains, without necessarily requiring crosses with foreign strains.

We presently report on the ongoing efforts to determine the genetic basis of salt-susceptibility in the Sabra experimental model of hypertension. The two standard strategies for detection of

Key words: hypertension, salt-susceptibility, candidate genes, epithelial sodium channel, arginine vasopressin, nitric oxide, sexual dimorphism, genome screen, microsatellites, linkage analysis.

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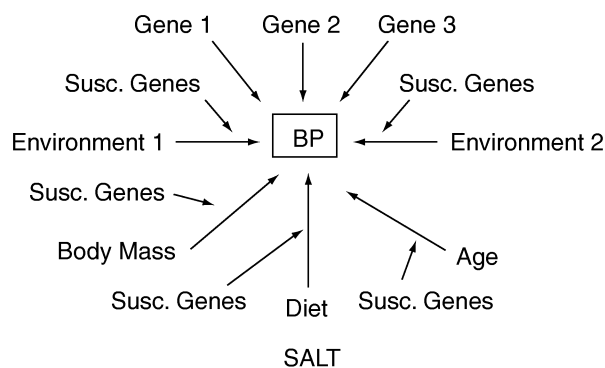


Fig. 1. Variables that affect blood pressure (BP).

disease genes are presented, the candidate gene approach and the steps taken so far in positional cloning.

CANDIDATE GENES FOR SALT-SUSCEPTIBILITY IN THE SABRA MODEL OF HYPERTENSION

Based on the known pathophysiological mechanisms of salt-susceptibility in the rat and published studies in the Sabra rat model of hypertension, a large number of candidate genes become relevant. The relevance of the nitric oxide system, the arginine vasopressin axis and the epithelial sodium channel are discussed below.

Nitric oxide system

Nitric oxide (NO) is a signaling molecule that is generated by the enzyme NO synthase (NOS). Three major isoforms have been identified: isoform I (nNOS) present in neuronal and epithelial cells, isoform II (iNOS) present in macrophages and vascular smooth muscle, and isoform III (eNOS) present in endothelial cells [14]. Expression of nNOS and eNOS is constitutive, whereas expression of iNOS is inducible. Among its diverse actions, vasodilation in particular renders NO a physiologically significant regulator of systemic and regional hemodynamics [14]. It is currently thought that resistance vessels are physiologically in a state of active dilator tone mediated by endogenous NO, and that NO acts as an important physiological regulator of normal BP [14]. Even though its role in the pathogenesis of essential hypertension remains controversial, there is a evidence to suggest a contributory role of NO in salt-induced hypertension. Chen and Sanders [15] have shown in the salt-sensitive Dahl rat fed 8% NaCl that by administration of L-arginine, which increases NO production, they prevented the development of hypertension. Administration of dexamethasone, which prevents expression of inducible NOS but not of constitutive NOS, along with L-arginine prevented the protective effect of L-arginine, suggesting that protection required activity of iNOS. Rees, Ben-Ishay and Moncada [16] studied the role of NO in the Sabra model of salt-susceptibility by inhibiting NO-synthase with L-NMMA and simultaneously administering a vasoconstrictor. They found a greater pressor effect in the salt-resistant than in the salt-sensitive strain, suggesting decreased NO generation in the latter leading to hypertension, and increased NO generation in former accounting for the resistance to the rise in BP. This conclusion was supported by a subsequent study by Lippoldt et al [17], who found that expression of inducible and neural NO synthase genes was lower

in SBH/y than in SBN/y before and after salt-loading. Of note is that gene expression of endothelial NO synthase (eNOS) was not different among the Sabra strains. This latter observation is of interest with regards to the role of NOS in salt-induced hypertension, as until now, the constitutive isoform III in endothelial cells has been considered to be the major regulator of arterial tone, at least under physiological conditions. On the other hand, salt-induced hypertension is a pathophysiological condition, and the inducible isoform II has been shown to play a role in vascular reactivity in a variety of pathophysiological conditions [14]. The NOS isoform I may influence vascular tone because of its presence in perivascular nerves and its central actions causing a decrease in sympathetic nerve traffic and BP [14].

Based on the above data, the NO system deserves to be considered a major candidate gene in salt-susceptible hypertension. Detection of the NO related culprit genes in salt-susceptibility, however, remains a very complex and tedious task. As pointed out by Dominiczak and Bohr [14], NO metabolism and actions involve at least eight significant intermediary steps, each of which may be in itself pivotal to the pathogenesis of salt-sensitive hypertension. Thus, although the NO system is unquestionably a worthy candidate gene, further extensive studies are needed at the molecular genetic level in the salt-sensitive models of hypertension before the NO-related genes can be directly implicated in salt-susceptibility.

The arginine vasopressin axis

Arginine vasopressin (AVP) has been studied extensively over the years with respect to hypertension. Although direct evidence for a central role of AVP in experimental hypertension is not abundant, the peptide has been proposed as a significant contributor to the pathogenesis of salt-related hypertension [18–20]. If AVP is indeed involved in salt-induced hypertension, the suggested major pathogenetic mechanisms are most likely an enhanced pressor effect of AVP and increased sodium retention in the salt-sensitive strain. AVP has been shown to modulate sodium reabsorption in the renal collecting duct [21]. One suggested mechanism for the latter effect of AVP is regulation of the epithelial sodium channel activity [22, 23]. Other possible mechanisms are a direct central nervous system effect of AVP on BP regulation [24] and an interaction of AVP with the sympathetic nervous system in the regulation of BP [19].

Experimental evidence for involvement of AVP in the pathogenesis of salt-induced hypertension in the Dahl rats is sound but not conclusive. In the Dahl model of hypertension, plasma AVP are similar at baseline in the S and R strains [25]. When the animals are exposed to high salt diet which leads to hypertension, plasma AVP levels rise more significantly in S than in R [25]. In one study, it was found that blockade of the pressor effect of AVP did not lead to a reduction in BP in the hypertensive S strain [25]. In contrast, in another study, an AVP V1A receptor antagonist given after 10 days of high salt-intake attenuated the rise in BP [26]. Finally, rats with diabetes insipidus failed to develop hypertension when loaded with DOCA-salt [20]. In the Sabra model of hypertension, a substantial body of evidence suggests involvement of the AVP system in salt susceptibility. Studies performed during the past decade have persistently shown disparate expression of the AVP gene among the salt-sensitive SBH/y and salt-resistant SBN/y strains. Enhanced expression of the AVP gene was found in the SBH/y strain at the level of the hypothalamus with greater

AVP mRNA content [27, 28], of the pituitary with greater AVP content [27], and of the peripheral circulation with increased AVP levels [28], compared to the salt resistant SBN/y strain. At the level of the kidney, increased activation of the AVP system was also found in the salt-sensitive strain, that is, in SBH/y, spontaneous urine excretion in prior to salt-loading was lower and urine osmolality higher than in the salt-resistant SBN/y rat [29].

Based on the above, the AVP complex appears to be established as a likely candidate gene for hypertension in the Sabra model of salt-susceptibility. As with the NO system, the continuing task involved in identifying the culprit genes within the AVP system is complex and very tedious, as numerous steps are involved in the metabolism and actions of AVP, all of which may affect BP regulation. So far, the AVP gene complex has not been differentially sequenced in the Sabra strains and no cosegregation studies of AVP genes with salt-induced hypertension have been published in the Sabra rats or the Dahl model of salt-susceptibility.

Epithelial sodium channel

The epithelial sodium channel (ENaC), which is found at the apical membrane of salt-reabsorbing epithelia in the kidney [30], is an important mechanism in the maintenance of salt-homeostasis. The protein is composed of three subunits: α , β and γ [30]. Its activity in the distal nephron is regulated by aldosterone and vasopressin [23]. Mutations of the β and γ subunits have been linked to spontaneous unregulated hyperactivity of the channel, leading to excessive renal sodium reabsorption and the development of hypertension in the clinical entity known as the Liddle's syndrome [31]. Mutations of the α subunit have also resulted in increased channel activity. Since genes that control sodium homeostasis are likely to fulfill a major role in salt-sensitive hypertension, the ENaC becomes a natural candidate gene for salt-induced hypertension.

In the Dahl model of hypertension, only a limited amount of work has been published to date on the potential role of the ENaC in the pathogenesis of salt-sensitivity. Of note are findings in a cross between Dahl S and Lewis rats in which linkage analysis detected one quantitative trait locus on chromosome 1 that could incorporate the genes for the β or γ subunits of ENaC [4]. In the Sabra model of hypertension, an effort was undertaken to detect mutations in genes encoding the subunits composing the ENaC. Grunder et al [22] sequenced the part of the genes encoding the cytoplasmic carboxyl terminus (C-terminus) of each of the three subunits, as the mutations leading to Liddle's syndrome have been found almost exclusively in that portion of the genes [31]. No differences were found in the sequences of the α or β subunits in SBH/y and SBN/y rats from the published sequences [22]. For the γ subunit, two identical polymorphisms were found in both SBH/y and SBN/y: the codon for the amino acid 573 was TGG instead of the published codon TGT, changing a Cys into a Trp and the codon for Cys 542 was changed from TGT to TGC without changing the amino acid [22]. Thus, SBH/y and SBN/y expressed mutations at the 573 and 542 sites, but there were no strain differences. Expression of the γ -C573W polymorphism in *Xenopus* oocytes did not alter ENaC activity [22]. This latter finding and the lack of difference between the salt-sensitive and salt-resistant strains render this particular mutation not likely to be related to the pathogenesis of salt-susceptibility in the Sabra model of hypertension. It is possible, however, that in other parts

of the ENaC mutations may be involved in the generation of hypertension. Mutations also outside the coding region, for example, in the promoter of one of the genes, could lead to misregulation of a subunit and altered activity of the sodium channel. A further possibility is that a mutant gene coding for a protein that is interacting with the ENaC to regulate its activity accounts for the development of hypertension. As ENaC is regulated by hormones such as corticosteroids and AVP [22, 23], the potential interaction of AVP with ENaC, two important candidate genes of their own right, becomes intriguing.

Additional studies are required to further explore the various components of the ENaC system as candidate genes for salt-susceptibility. The recent localization of the subunits of ENaC to chromosomes 1 and 4 in the rat [22, 32] and the ever increasing density of the rat genome map call for linkage studies that should help resolve the role of the ENaC system as a major candidate gene for salt-susceptibility.

Conclusions

As in other experimental models of hypertension, the direct deductive candidate gene approach has not yielded so far the culprit genes for salt-susceptibility in the Sabra model of hypertension. This may not be surprising, as the candidate gene approach is severely confounded by the deep complexity of each candidate gene, which in fact involves an entire system complex, a fact that is not commonly appreciated. Each candidate gene may be affected by a number of interim steps that involve among others its synthesis, attachment to action site, activation of secondary pathways, and metabolism. Each one of these steps may be the factor determining salt-susceptibility. Thus, straightforward sequencing a portion of a candidate gene may be only a preliminary step in a very lengthy and tedious process of attempting to prove the relevance of the candidate gene to the pathogenesis of a clinical entity. Combined with positional cloning, however, the candidate gene approach may be a more sensible methodological approach to the problem, with better chances of success in identifying the salt-susceptibility genes. As in the case of the "ob" gene in the mouse, however, it is also possible that the genes for salt-susceptibility may not be part of known pathophysiological mechanisms of hypertension and that new, yet unidentified responsible proteins may be uncovered in the deductive positional cloning approach. Once such new peptides are uncovered, they become candidate genes, and their pathophysiological relevance to salt-susceptibility remains to be established.

GENETIC MAPPING OF SALT-SUSCEPTIBILITY GENES

As the first strategic steps undertaken in the multi-stage process of positional cloning of salt-susceptibility genes in the Sabra rat model of hypertension, genetic mapping to chromosomal regions was carried out in cross-bred SBH/y and SBN/y rats [33]. The BP response to salt-loading was determined in the F2 generation, the mode of transmission of salt-susceptibility was investigated, and cosegregation studies of BP with microsatellite markers spread throughout the rat genome and linkage analyses were performed.

Cross-breeding

Sabra hypertension-prone (SBH/y) and hypertension-resistant (SBN/y) rats from the Barzilai Medical Center colony [13] were cross-bred [33]. The initial cross (Cross 1a) was between 7 female SBH/y rats and 2 male SBN/y rats. To ensure reproducibility, this

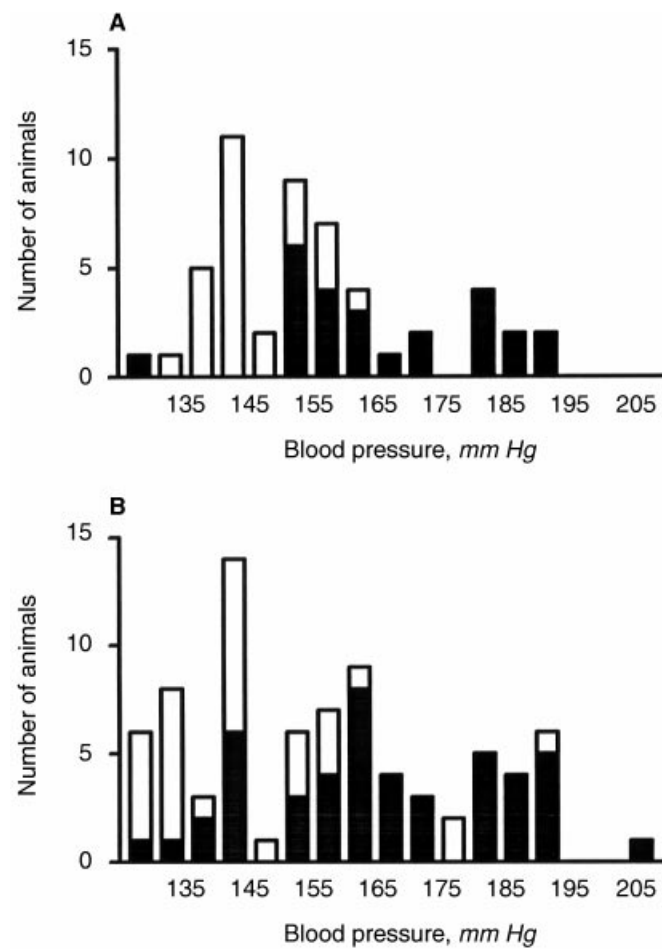


Fig. 2. Blood pressure (BP) distribution after salt-loading with DOCA-salt in F1 cohort of cross 1 (A) and cross 2 (B). Symbols are: (□) female; (■) male.

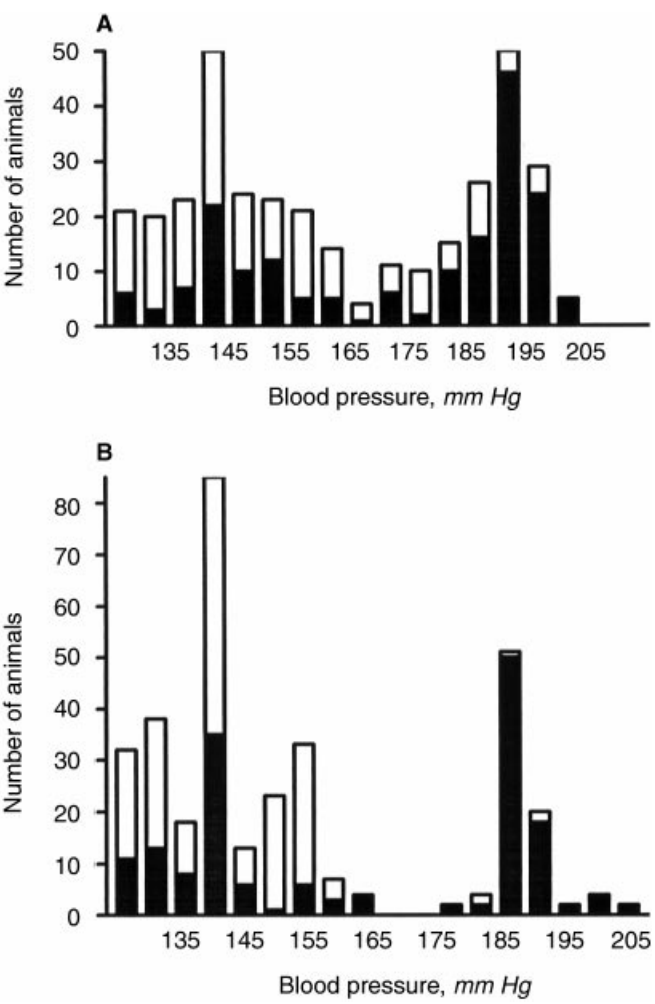


Fig. 3. Blood pressure (BP) distribution after salt-loading with DOCA-salt in F2 cohort of cross 1 (A) and cross 2 (B). Symbols are: (□) female; (■) male.

cross was repeated (Cross 1b) between 3 female SBH/y rats and 1 male SBN/y rats. The reciprocal cross (Cross 2a) was between 3 female SBN/y and 1 male SBN/y rats and repeated (Cross 2b) between 5 female SBN/y rats and 2 male SBN/y rats. The F1 progeny from each cross were inbred (brother-sister mating) to produce F2 generations. The F2 populations were weaned at one month of age. The phenotype of interest, BP response to salt-loading, was determined by measuring basal BP at six weeks of age and again after four weeks of treatment with DOCA-salt, as previously described [13].

Mode of transmission of salt-susceptibility

Analyses of the pedigrees leading from the parent progenies through F1 to the F2 generations uncovered a highly unusual and complex mode of transmission, as detailed below:

Cross 1. In cross 1 (female SBH/y with male SBN/y), the BP response to salt-loading of the F1 cohort ($N = 51$) was unimodal, with a widespread distribution throughout the BP scale (Fig. 2A). Analysis after stratification by sex revealed a different BP distribution between females ($N = 26$) and males ($N = 25$), females having generally a lower BP response than males. In the F2 cohort ($N = 389$), a bimodal BP distribution was found with a peak at the lower range of the scale of the BP response (no hypertensive

	Cross 1	Cross 2
	(F-SBH/y x M-SBN/y)	(M-SBN/y x F-SBN/y)
P	XhXh x XnY	XhY x XnXn
F1	XhXn,XhY	XhXn,XnY
F2	XhXh,XnXn	XhXn

Fig. 4. Scheme of proposed Mendelian mode of transmission of the salt-resistance n-allele in crosses 1 and 2, accounting for the rise in blood pressure (BP) after salt-loading in females in cross 1 and the lack of hypertensive response in cross 2.

response) and another peak at the higher end (hypertensive response; Fig. 3A). The bimodality was explained in part by differences in BP distribution between males ($N = 179$) and females ($N = 210$): About 2/3 of the males tended to have a BP response in the higher range and 1/3 in the lower range, whereas 2/3 of the females had a BP response in the lower range and 1/3 in the higher range.

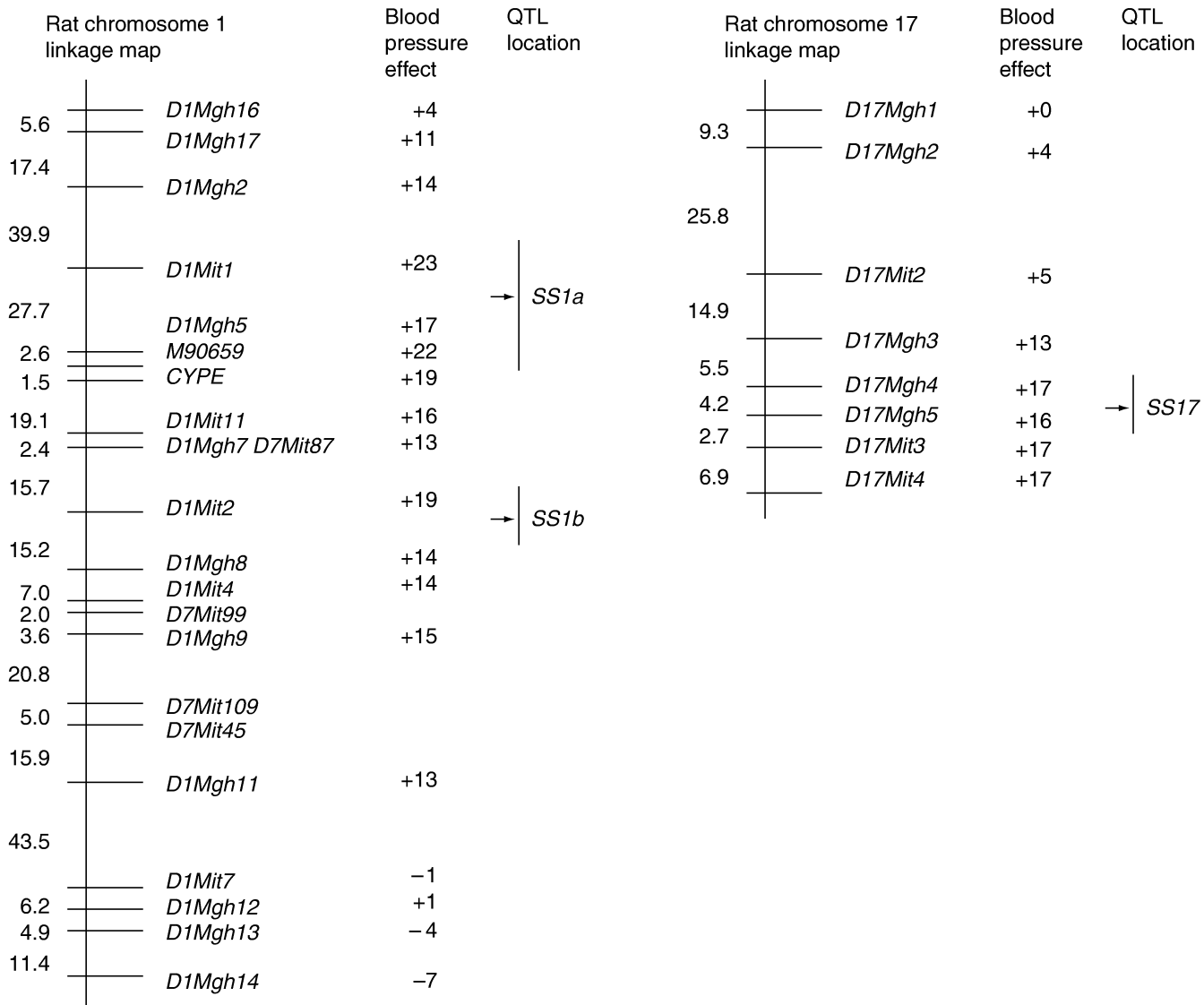


Fig. 5. Linkage map for rat chromosome 1 based on data from 232 rats (100 male and 132 female - overall effect). The blood pressure (BP) effect was calculated as the difference in BP between the homozygous for the H (salt-sensitive hypertensive) allele and those homozygous for the N (salt-resistant normotensive) allele. The arrow indicates the maximum LOD score and the QTL vertical bar indicates ± 1 LOD for linkage to BP.

Cross 2. In Cross 2 animals, the reciprocal cross (male SBH/y with female SBN/y), the BP response to salt-loading in F1 ($N = 78$) was unimodal as in Cross 1 (Fig. 2, lower panel). Stratification by sex revealed that BP in females was in the low range response in most animals (28 of 31), whereas in males the BP spread evenly throughout the scale. In the F2 cohort ($N = 338$), BP distribution was bimodal (Fig. 3B), similar to that found in Cross 1. After stratification by sex, the BP response in males ($N = 167$) was found bimodal, with about half of the animals having a lower response and half having a hypertensive response. Females ($N = 171$) had nearly all BP measurements ($N = 166$) in the low range.

This complex and highly unusual mode transmission of the BP response to salt-loading in the F1 and F2 generations in these crosses is presently difficult to interpret. The bimodal distribution of BP and sexual dimorphism are, nonetheless, highly suggestive of the following three possibilities: (1) That at least two major

separate salt-susceptibility autosomal genes must be involved among the sexes, one accounting for salt-sensitivity (the hypertensive response) and the other for salt-resistance (the lack of a hypertensive response); (2) different sets of autosomal genes may account for salt-susceptibility in males and females; (3) a major salt-resistance dominant allele on the X chromosome modulates expression (inhibitory effect) of the salt-sensitive autosomal genes in females. The salt-resistance allele on the X chromosome appears to be transmitted in a pattern of Mendelian dominance (Fig. 4), since in the presence of an allele for resistance on Chromosome X in F1 of crosses 1 and 2 in all females (X_n), there was a lack of hypertensive response. In contrast, in F2 of cross 1, females could develop hypertension as only some but not all carried the resistance allele, thus allowing the autosomes to come into action, whereas in cross 2, all females carried the N allele and thus BP did not rise in response to salt.

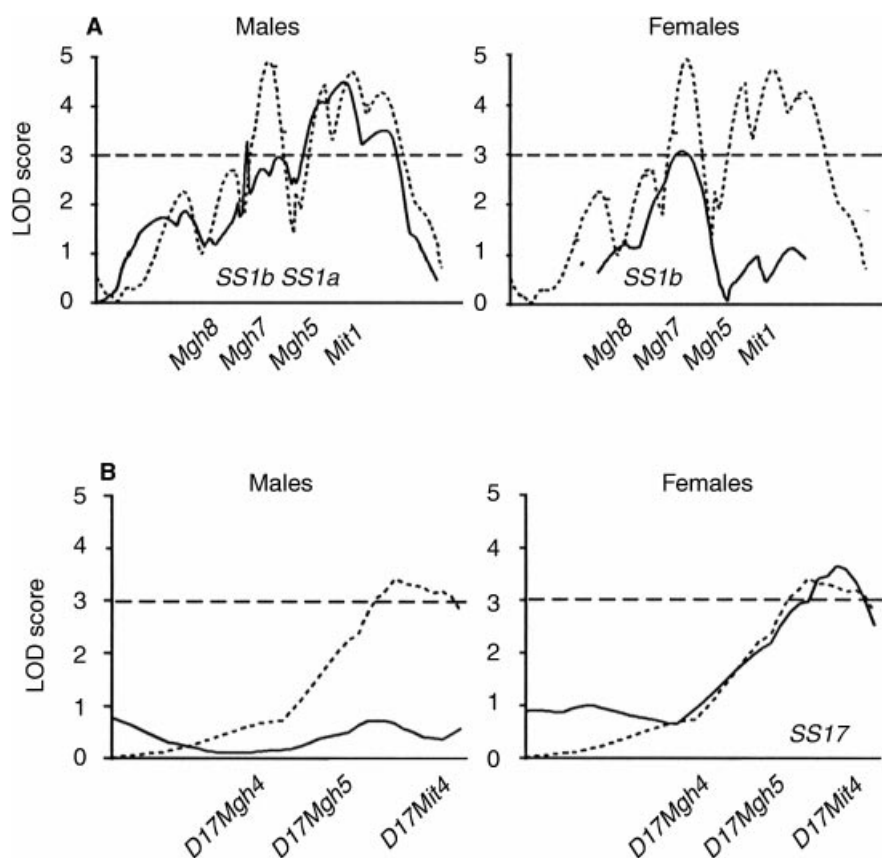


Fig. 6. Tracing of LOD score along chromosomes 1 (A) and 17 (B) for blood pressure (BP) after salt-loading as generated by MAPMAKER/QTL, based on an analysis of the F2 cohort. The dashed horizontal lines represent LOD = 3. The dotted curved lines in the lower panels represent the entire cohort analysis upon which the LOD scores by sex are superimposed as continuous curved lines.

Genetic mapping

Random genome-wide screen was performed in the Sabra model of hypertension with 210 microsatellite markers distributed over the 20 rat autosomes and covering 96.8% of the rat genome [33]. Genotyping was carried out as previously described [13]. The data were analyzed to determine if BP after salt-loading cosegregated with markers on chromosomes 1 to 20, and multipoint linkage analyses were performed using the MAPMAKER/EXP 3.0 and MAPMAKER/QTL 1.1 programs [34, 35]. As the mode of inheritance of salt-susceptibility in this cross was found highly complex (see section above), the free default model was used for calculations. The resulting analyses showed that BP after salt loading cosegregated significantly with well defined regions on chromosomes 1 and 17. There was no cosegregation with markers on any of the other autosomes [33]. Analyses of chromosome X data are still ongoing.

Chromosome 1. Analysis of the data from the entire F2 cohort detected two QTLs on chromosome 1 (Fig. 5). One, designated *SS1a*, with a peak LOD score of 4.71 and a span of 64.9 centimorgans (cM; Max LOD score ± 1), accounted for 43% of the difference in the BP response to salt-loading between SBH/y and SBN/y. The second QTL, designated *SS1b*, has a peak LOD score of 4.91 and a span of 17.2 cM, and accounts for 35% of the difference in the BP pressure response between SBH/y and SBN/y. Repeat analysis of the data after stratification by sex revealed sexual dimorphism (Fig. 6). In males, two QTL were detected, one coinciding with *SS1a* with a peak LOD score of 4.52 and accounting for 59% of the difference in the BP response, and

the other coinciding with *SS1b*, with a peak LOD score of 2.98 and accounting for 44% of the difference in BP response to salt-loading. In females, only one QTL was detected on this chromosome, coinciding with *SS1b*, with a peak LOD score of 3.08, a span of 18 cM, and accounting for 30% of the difference in the BP response to salt-loading between SBH/y and SBN/y.

Chromosome 17. Analysis of the data from the entire F2 cohort detected one QTL designated *SS17*, with a peak LOD score of 3.43, a span of 15.8 cM and accounting for 31% of the difference in the BP response to salt-loading between SBH/y and SBN/y. In males, repeat analysis after stratification by sex revealed no cosegregation of any region on chromosome 17 with the BP response to salt-loading, whereas in females one QTL was detected, coinciding with *SS17*, with a peak LOD score of 3.66, a span of 16.3 cM, and accounting for 38% of the difference in the BP response to salt-loading between SBH/y and SBN/y.

Comments

Genetic mapping has thus far allowed the identification of two loci on chromosome 1 and one locus on chromosome 17 as carrying genes that allegedly contribute to the genetic variance of the BP response to dietary salt intake [33]. The two QTLs on chromosome 1, *SS1a* and *SS1b*, are about 20 cM apart, do not overlap, and most likely represent two distinct gene loci. The one QTL on chromosome 17, *SS17*, is likely to represent a third gene. Thus, salt-susceptibility in the Sabra rats appears to map to three distinct gene loci on chromosomes 1 and 17. Comparison of these findings with respect to salt-susceptibility related gene loci in

crosses involving the Dahl rats reveals intriguing parallels. In male hybrids from a cross of the Dahl SS with the Lewis rats (LEW/NCrLBR), two QTLs were detected on rat chromosome 1 [4] and one on chromosome 17 [5]. The localization of the QTLs on chromosome 1 at *cytochrome P450 2B2* [4, 36] and *SA* [4, 37] loci correspond well with the placement estimates of *SS1a* and *SS1b*. The QTL detected in the cross of the Dahl SS with the Lewis rats on chromosome 17 [38] also corresponds well with the placement of *SS17*. The sexual dimorphism in the genetic basis of salt-susceptibility that was uncovered in the Sabra model is novel. In the male rats, two culprit QTLs were localized to chromosome 1, coinciding with *SS1a* and *SS1b* and in the female rats, one QTL was detected on chromosome 1 coinciding with *SS1b*, and a second QTL on chromosome 17. These findings suggest that *SS1a* is sex specific for males, that *SS17* is sex specific for females, whereas *SS1b* is not sex specific. If the effects of the two genes encoded within these QTLs are additive, in male Sabra rats, the BP response to salt-loading can be entirely accounted for by the two QTLs detected on chromosome 1, whereas in the female Sabra rats, chromosomes 1 and 17 together contribute to nearly 70% of the BP response to salt-loading. Thus, while the combined effects of *SS1a* and *SS1b* account for a large portion of the overall phenotype variance in male rats, it would appear that other, yet unidentified genetic loci must contribute additionally to the genetic variance in females, indicating the need to pursue the search for additional gene loci at other chromosomal locations. The sex specificity with respect to the effects of QTLs *SS1a* and *SS17* implies that different sets of autosomal genes may contribute to salt-susceptibility and hypertension among either sexes. Such sex specificity of QTLs is consistent with reports in other strains of genetically hypertensive rats [39]. While epistatic and ecogenetic interactions of genes with the Y and X chromosomes may come into play, hormonal interaction at the transcriptional as well as post-translational level represent an alternative possibility that needs to be addressed in future studies by including appropriate hormonally and surgically modified experimental groups. Finally, genetic mapping as part of the overall strategy of positional cloning leads eventually to identification of candidate genes, whether known or novel proteins. The large number of potential candidate genes already identified in the areas swept by the placement confidence intervals for *SS1a*, *SS1b*, and *SS17*, virtually precludes further evaluation of the present results with respect to gene identification, until higher resolution linkage information becomes available. Such information should be forthcoming from congenic substrains currently under construction. The congenic strains will also help clarify the significance of the identified QTLs and determine if they indeed encode the putative genes for salt-sensitivity or for resistance in terms of the development of hypertension.

SUMMARY

The current search for salt-susceptibility genes in the Sabra model of hypertension has involved the deductive candidate gene approach and the inductive positional cloning scheme, consisting of random screening of the entire rat genome for the culprit genes and chromosomal mapping. The candidate gene approach has focused on several interesting candidate systems, including the nitric oxide system, arginine vasopressin, and the epithelial sodium channel complex. Each system-complex involves a large number of peptides and reactions, and each of these could be

involved as an intermediary step in the genesis of salt-susceptibility. The likelihood of detecting the actual responsible genes for salt-susceptibility using this methodology alone is thus low and the process, however important, is slow and high risk in terms of cost-effect. Forthcoming efforts will most likely need to combine the candidate gene approach with positional cloning. The initial step in positional cloning consisting of genetic mapping has led us, as an interim step, to investigate the mode of transmission of salt-susceptibility, which we found highly unusual and complex, suggesting involvement of at least two sets of autosomal genes that may be different in male and females, and the presence of an inhibitory allele on the X chromosome in females. Using linkage analyses, we detected two QTLs for salt-susceptibility on chromosome 1, and one QTL on chromosome 17. We found sex specificity with one QTL specific for males only, one specific for females only, and a third involving both males and females. Thus, different susceptibility genes for hypertension might come into play between the sexes in the Sabra model of salt-susceptibility. Even though it may be reasonable to interpret our findings in a more general sense, it is important to stress that our findings apply only to the specific strains and experimental conditions studied. Thus, extrapolations to other models, and certainly to human traits, should be made with great caution. It is apparent, nonetheless, that further exploration of the Sabra model of salt-susceptibility, the eventual identification of causative genes, and the decoding of the mechanism of action in this experimental model, may ultimately provide clinically relevant tools for novel diagnostic and therapeutic approaches that will benefit an epidemiologically important group of patients with hypertension.

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